EFFECTS OF EXTERNAL ELECTRIC FIELDS ON LIGHT TRANSMITTANCE IN ISOLATED CRAYFISH NERVES

THESIS

Presented to the Graduate Council of the
University of North Texas in Partial
Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Brian S.W. Northcutt, B.A.

Denton, Texas

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Acute effects of a pulsed external electric field (PEEF) at 20 V/cm and a d.c. EEF at 90 V/cm on light transmittance in an isolated compound crayfish nerve was measured. In a third series, the nerve was pre-treated with the Na^+ channel blocker tetrodotoxin (TTX). A PEEF produced an irreversible increase in the variation of light transmittance in normal nerves but a reversible increase in TTX treated nerves. This data was statistically insignificant. The d.c. EEFs produced a reversible and statistically significant enhancement of variation in light transmittance in both untreated and TTX-treated nerves. The findings may be due to either (1) an alteration in the ion/fluid flux within the nerve or (2) a physical alteration of protein molecules in the membranes.

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INTRODUCTION

It has been clearly established that there is evidence that external electric fields(EEFs) have certain effects on living matter and on non-living organic matter. One major site of action of the EEF seems to be at the membrane of the cell. Research as far back as 1910 has been geared toward explaining the effects of fields at the membrane level(1). This would make sense considering the high lipid concentration of the membrane that provides a non-conductive barrier between the extracellular and intracellular field strengths. Pulsed EEFs have been shown to cause enhanced reuptake of calcium by bone in the healing process(2), stimulate collagen protein synthesis, and inhibit cAMP accumulation stimulated by parathyroid hormone(3). Also consistent with the theory of membrane effects is the increased binding of activating ions on the sodium and potassium ATPase surface in low frequency fields(4). The results of that study suggested a 5 to 15 percent increase in enzyme activity around 60Hz. The effect of changes in surface ions may be reflected in a study that reported enhanced amplitude of action potentials in isolated frog nerves during exposure to EEFs for 30 to 60 minutes at

strengths of 45 and 90 V/cm(5). A question that arises is what structural changes, if any, occur at the membrane surface in the presence of an EEF. Goodman et al. used a cell surface chromatography technique to show that there are subtle changes in cell surface composition and charge during exposure to pulsed magnetic fields (6,7). More recently it has been shown that natural levels of biological fields cause concentration gradients to form among lipids in binary mixtures(8) and that fields in the range of 40 to 240 V/cm stimulate ion emission from a liquid sample directly into a vacuum through a synthetic membrane containing around one million channels per square centimeter(9). These studies suggest the existence of a combination of structural changes and changes in ion flow at the level of the membrane. Studies have shown thresholds as small as 5uV/cm at 25 to 200 Hz (10) can produce these types of effects. Fields of much higher magnitude have been used with caution since such fields may produce thermal heating. How effects are caused on membranes that are exposed to EEFs has been the subject of much of the research done recently and for the past couple of decades. Possible reasons for structural and functional changes include enhancement of transmembrane voltage, electroconformational changes, electropermeablization (of membranes), electrolesion, electroporation, and electrostimulation of metabolic

processes(11,12).

EEF types that have been used include alternating, direct current, and pulsed fields. The pulsed fields, as previously described, have been implicated in numerous biological processes including higher breast cancer rates among male utility workers(13), membrane transport(13), tumor promotion(15), and even increased rates of subjective thinking(16). It is because of these past results that pulsed EEFS have been chosen as one of the experimental parameters used in this study. Research that suggest ion movement due to reported effects of EEFs on electroosmolarity is the reason that a direct current field has been chosen as another experimental parameter. Finally, to test for effects of EEFs on ion flux within or across the membrane, it was decided to use tetrodotoxin(TTX) to block sodium channels.

Numerous workers have used changes in optical properties of nervous tissue as a criterion in studying the effects of various conditions and agents on the structural properties of membranes(17,18,19). Among the techniques available for measuring changes in optical properties is visible absorption spectroscopy. Visible absorption spectroscopy is the process by which a light is applied to a substance and the beam transmitted at the other end is measured. When molecules in the substance are moved to a

higher energy state due to the energy of the beam, they are said to have absorbed this energy. Molecules of different structures generally absorb light energy in different quantities. If the structures in a biological tissue change in response to an EEF, then it should be possible to detect these changes with a photocell(20). The specific technique that was employed in this study was one that measured the variation of transmittance. Transmittance is generally 1 minus the absorbance, however, a certain amount of light scattering is naturally involved since photons may be deflected from a direct path. So for the purposes here, transmittance will be taken to mean the amount of light that was actually passed on to the transducing device. This technique has been done on the living tissue of the Electrophorus electric organ during activity(17), on changes in crab nerves during action potentials(18), and on changes in squid giant axon during action potential(19). With the prior research on membrane level effects combined with the precedence for structural changes as measured by optical techniques, this method was selected for the experiments in this study.

The purpose of this study therefore was twofold: (1) To measure the effects of an EEF on an optical property, light transmittance, in compound nerves of crayfish and (2), to measure the effects of an EEF on light transmittance in

nerves that had been treated with TTX, a well known Na^+ channel blocking agent. The purpose of the former was to study EEF effects on the general structural integrity of the nerves while the latter would involve the effects of EEFs on ion(Na^+) flux in the membranes.

METHODS

A total of 60 crayfish were used in this study. Live crayfish were housed in four separate 10 gallon freshwater aquariums for three to twenty days prior to the experiment. There were 10 to 12 specimens per aquarium and water temperature was the same as room (22-24°C). Crayfish ranged in length from 9 cm to 14 cm. The ventral nerve cords removed ranged from 3.2 to 5.1 cm in length with a diameter of 0.6 to 1.0 mm. There are two giant nerve fibers in this nerve, and 200-300 smaller axons originating from 6 ganglia located along its length. The ventral nerve cords were removed through dorsal entry. The tail muscle was lifted up and the nerve teased away from other tissue while carefully snipping the collaterals. They were then placed in a petri dish containing amphibian Ringers solution (pH=7.4) for a total of 15 minutes in order to overcome possible surgical shock. In the TTX experiments, the nerves were first placed in Ringers solution for 14 minutes then the solution was replaced with Ringers solution containing a 10mM concentration of TTX (pH=7.4). The treatment time for TTX was 1 minute to make a total equilibration time of 15 minutes.

The basic premise of the experimental technique was the measurement of changes in light intensity of a beam of white light passing through a crayfish nerve. The changes were so small as compared to the noise of random scattering and transmittance of photons, several steps were taken to increase the signal to noise ratio. First, the nerve and light source were enclosed in a flat black painted box in order to eliminate all outside sources of light. Second, the apparatus was enclosed in a copper Faraday cage to reduce electronic noise in the transducer leads and to zero out external environmental electric fields. Third, the light source drew current from a regulated power source to eliminate fluctuations in intensity due to power surges. Fourth, all wires were grounded to prevent stray magnetic or electrical fluctuations in the wire's currents. Nevertheless, there was still a very small signal to noise ratio. It was decided to use signal averaging which was built into our oscilloscope. The overall change in intensity during any one experiment was less than 2% and therefore the measurements taken were within this same 2% window and overall changes in absorbance were not calculated. Six different mathematical measurements of the resulting waveforms were considered at averages of 64 sweeps per measurement. These included root mean square, amplitude, minimum, maximum, positive duty cycle, and

negative duty cycle.

Light transmittance measurements were taken using an apparatus constructed by combining several commercial and non-commercial manufactured parts similar to that constructed by Cohen et al.(18) and by Newman(20). The basic design was to direct a beam of light through the nerve, and onto a transducer that would measure the intensity of the light. The transducer used was an RCA 4409 phototube removed from a Bausch and Lomb Spectronic-20 spectrophotometer. The leads from the tube were made from grounded coaxial cable and went directly back into the spectrophotometer housing as if the tube itself were only given an extension cord for use outside of the housing. The meter of the spectrophotometer was bypassed and redirected to a Tektronics TDS 320 oscilloscope in order to detect extremely rapid changes in waveform patterns(<20ms). The phototube was mounted in the place of an eyepiece on a standard lab microscope. Light from the microscope was directed upward through a 0.4mm diameter hole located on the stage, through the nerve, out of the chamber's transparent window, and to the phototube. The bulb used was a 50W tungsten filament with a UV filter that produced 34,450 Lumens at peak efficiency. On the stage was a chamber consisting of four major parts, 1) a transparent plastic foundation implanted with four agar coated silver wire

electrodes, 2) a black plastic chamber cover fitted with 3) a transparent plastic window , and 4) an agar coated aluminum electrode(see Fig. 1a). These four parts served as the experimental chamber for each experiment. The chamber was lined with moistened filter paper to prevent the nerve from drying. The chamber measured 3.0 cm wide x 5.5 cm long x 1 cm tall for a total area of about 16.5 cm^2. The field generating electrode was lateral to the nerve and measured 1 cm x 5.0 cm. The phototube was placed 3 cm over the chamber resulting in a focal distance of 4 cm over the nerve. The temperature of the chamber ranged between 22 and 24 degrees Celsius. No measurable heating effects of the light were seen for periods of up to 15 minutes. Since the entire measurement period was only 192 seconds, significant heating of the chamber was assumed to be negligible.

The chamber was part of a collection of apparati depicted in Figure 1b. This apparatus consisted of the chamber / microscope / phototube complex. A dark box, which was a flat black painted cardboard box, was used to block out outside light. The dark box measured 32.5 cm wide x 37 cm long x 32.5 cm tall. The Faraday cage measured 50 cm wide x 61.5 cm long x 55 cm in height.

The entire setup is shown in schematic form in Figure 2. The four agar coated electrodes located on the stage were used to determine viability of the nerve. These were

supplied by four leads, two stimulating and two recording. Nerve stimulation was supplied by a Grass SD9 stimulator. The resultant action potential was displayed on a Tektronics 550 oscilloscope as described by Aidley(21). A double lead connected the phototube to the amplifier portion of the spectroscope and another double lead supplied the power to the light source. The EEF was supplied through a single lead connecting the agar-coated aluminum electrode in the chamber to the positive terminal of a Grass S48 stimulator. All components were commonly grounded.

After removal and equilibration, a nerve was placed in the chamber and allowed to equilibrate further for at least 5 minutes. At t_o, sixty-four sweeps gated to 20ms following the initiation of the sweep were taken at the rate of one sweep per second. The same sweep measurement was taken at t=64 seconds and t=128 seconds. Therefore, each experiment consisted of a 64 second pre-exposure, a 64 second exposure, and a 64 second post-exposure period for a total of 192 seconds. The sham-treated control nerves were not exposed to any EEF. Pulsed field-treated nerves were exposed to a d.c. pulse of 5ms in duration at the rate of 1 per second for 64 seconds. The pulse triggered the measurement. D.C. exposed nerves were exposed to a continuous 90 V/cm field from t=64 seconds to t=128 seconds with measurements being taken at the rate of one per second. The experiments were

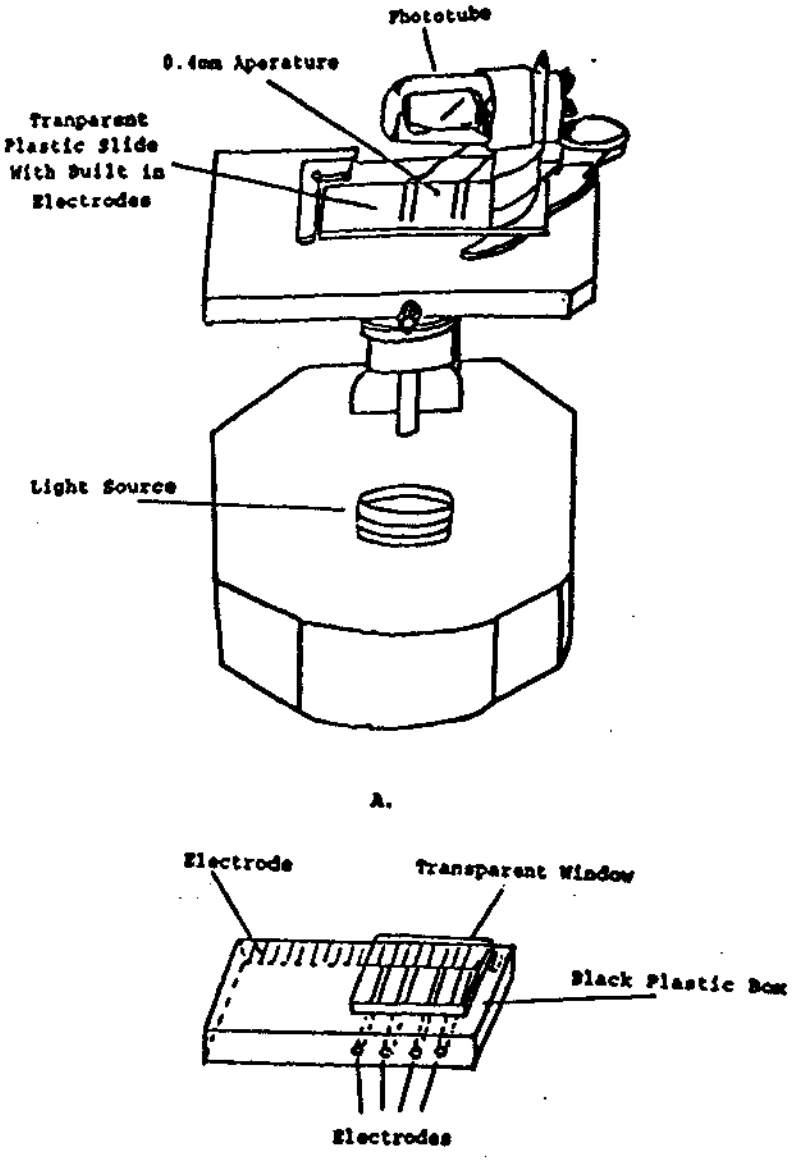

Figure 1: A: Apparatus used to measure light transmittance in crayfish nerves.

B: Chamber used to house crayfish nerves during light transmittance experiments.

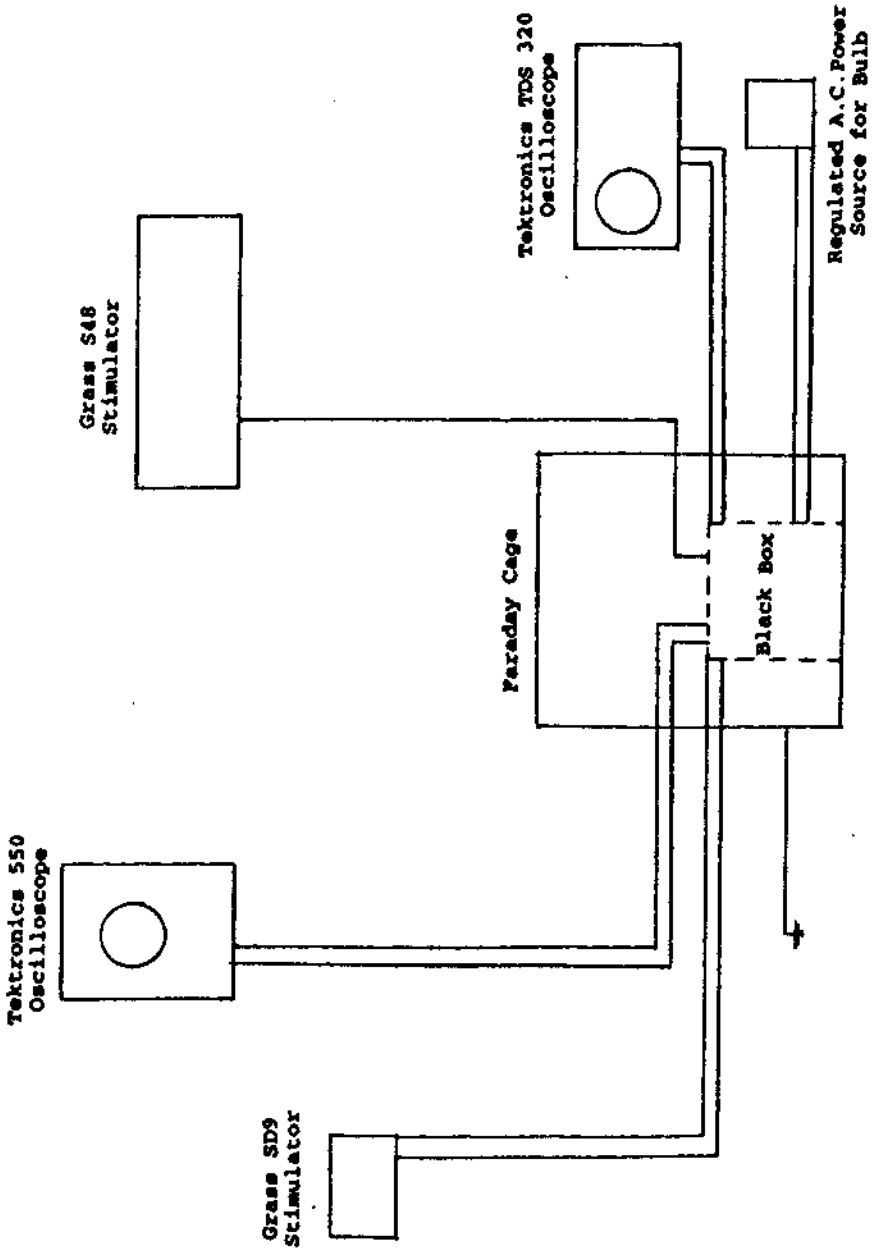

Figure 2: General schematic of apparatus used to measure light transmittance in crayfish nerves.

divided into a sham-treated control series and a test series as follows:

A: Sham controls, no field,

B: TTX treated, no field,

C: Pulsed field exposure,

D: Pulsed field exposed plus TTX(10mM) treatment,

E: D.C. field exposure,

F: D.C. field exposed plus TTX(10mM) treatment.

RESULTS

Figure 3 shows oscilloscope tracings during a typical single sweep. The wave is erratic and clearly shows the amount of noise that was dealt with. However, dimensions of the waveform were available through digital computer analysis. The data from the sixty nerves used are given in figures 4 through 9 in the form of bar graphs. The data were analyzed using Tukey's Test(22). This statistical test, a variation of ANOVA, compares group means and determines which specific means are different from those in the same treatment group. A summary of the results are depicted in Table 1. Since changes in the positive duty cycle are used to reflect changes in light transmittance, a brief explanation is warranted. The amount of light that was passed on to the phototube represents the degree of transparency of the nerve, that is, the more light that reaches the phototube, the more transparent the nerve. The actual change in the amount transmitted in the direction of the phototube was 0 to 2% of the total amount of light or near 689 Lumens which is consistent with the work of Cohen et al.(18). Waveform analysis using signal averaging was used to discern consistent patterns in this light

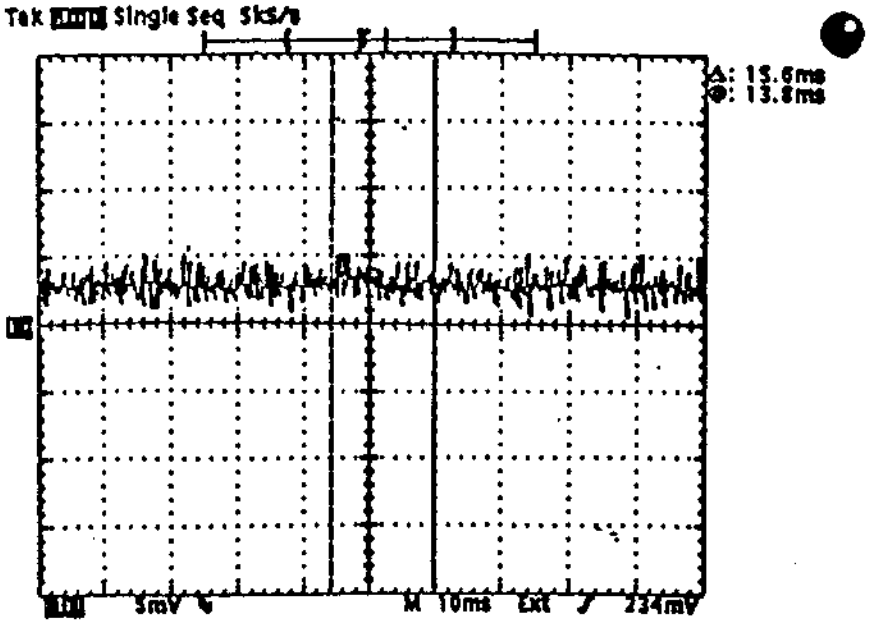

Figure 3. Oscilloscope tracing from a typical run on light transmittance in isolated crayfish nerves.

transmittance. The quantitative representation of this degree of change is given in the positive duty cycle (PDC). The PDC is the ratio of the positive pulse width to the signal period expressed as a percentage (i.e. Positive Duty Cycle = Positive Width/Period x 100%). PDC changes as the variation in light that reaches the phototube changes. This measure that was used to determine the rapid changes in the transparency of the nerve during the course of each experiment.

Figure 4 illustrates the mean PDC of 10 sham-exposed nerves over a 192 second time period. There was no significant change in the light transmittance of the nerve during the allotted time.

Figure 5 depicts the mean PDC of 10 sham-exposed nerves treated with TTX. There were no statistically significant changes in light transmittance by the nerve during the test period.

Figure 6 represents the mean PDC of 10 nerves exposed to a pulsed EEF of 20 V/cm, 5 ms duration, at the rate of 1 pulse per second (pps). An initial increase of 10.52% was evident during the exposure period which was continued during the post-exposure period to a lesser degree (3.65%). These changes, however, were found to be statistically insignificant.

Figure 7 depicts the response of 10 TTX-treated nerves

to a pulsed EEF of 20 V/cm, 5 ms duration, at the rate of 1 pps. An initial increase of 11.51% of the PDC was noted during the exposure period followed by a 15.29% decrease during the post-exposure period. These findings, however, were not statistically significant.

Figure 8 depicts the mean response of 10 nerves to a continuous d.c. EEF of 90 V/cm. The increase observed during the exposure period was 20.54%. This was statistically significant from the pre-exposure period ($p<0.05$). During the post-field phase, however, a decrease of 20.93% in the PDC occurred which was statistically significantly different from the pre-exposure and exposure periods ($p<0.05$). These findings indicated a distinct optical change in the membrane of the nerves.

Figure 9 illustrates the same d.c. field treatment on 10 TTX-treated nerves. An initial increase in PDC of 25.38% was observed during the exposure period. This was followed by a 30.63% decrease. The changes observed during the exposure period again were statistically significant from the pre- and post-exposure periods ($p<0.05$) and indicative of changes in the nerve.

A summary of the data is given in Table 1. These data indicate that:

(1) Nerves exposed to pulsed EEFs of 20 V/cm, 5 ms duration, at 1 pps failed to exhibit any significant changes in light

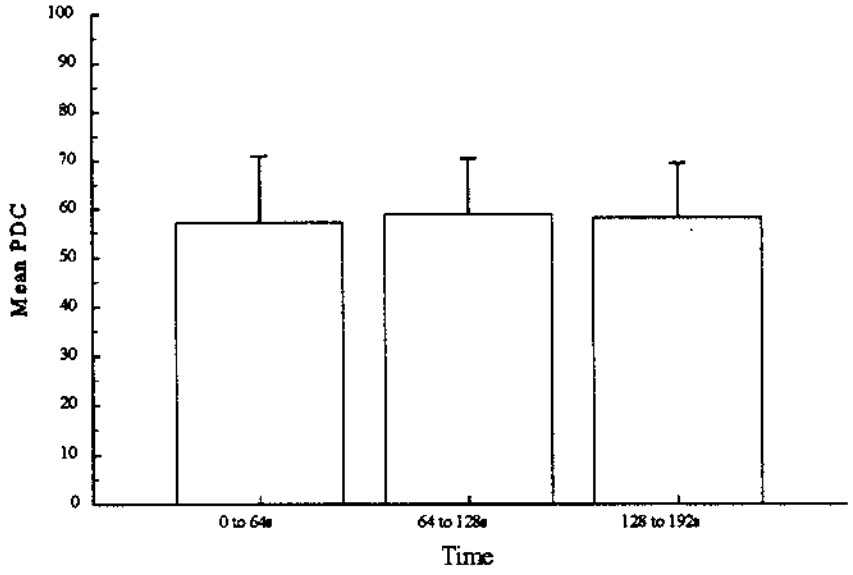

Fig. 4 Mean variation in light transmittance (PDC) in 10 isolated sham-treated crayfish nerves over a period of 192 seconds.

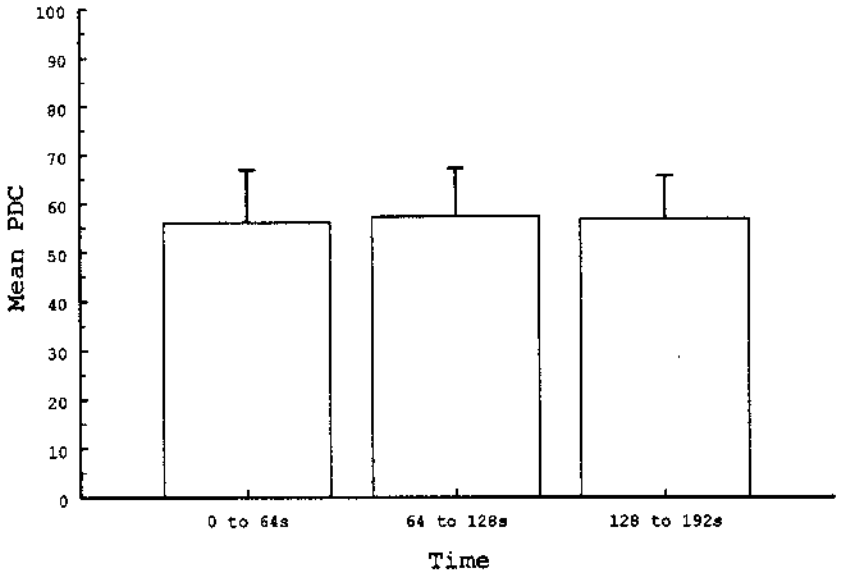

Fig. 5. Effects of 1 minute pre-treatment with TTX (10mM) on the mean variation in light transmittance (PDC) in 10 isolated crayfish nerves.

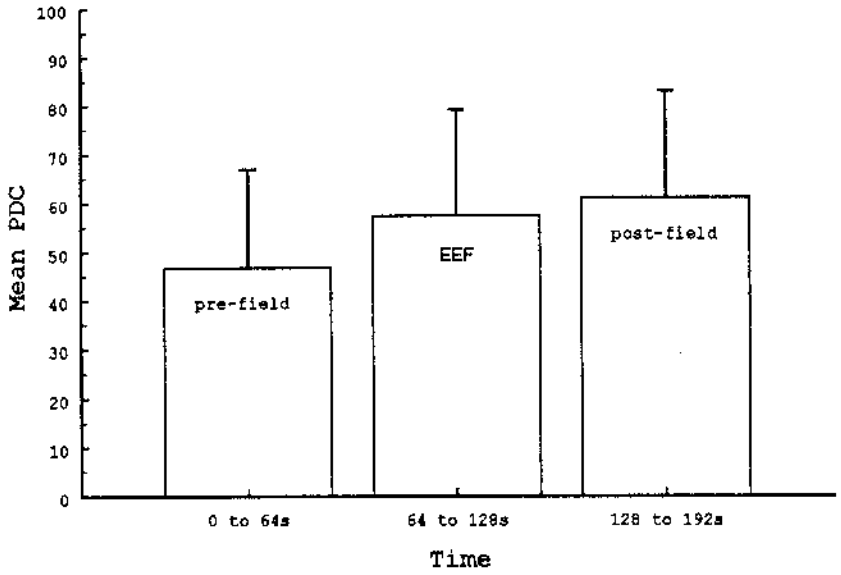

Fig. 6. Effects of a pulsed EEF (20V/cm, 5ms duration, at 1 Hz) on the mean variation in light transmittance (PDC) in 10 isolated crayfish nerves.

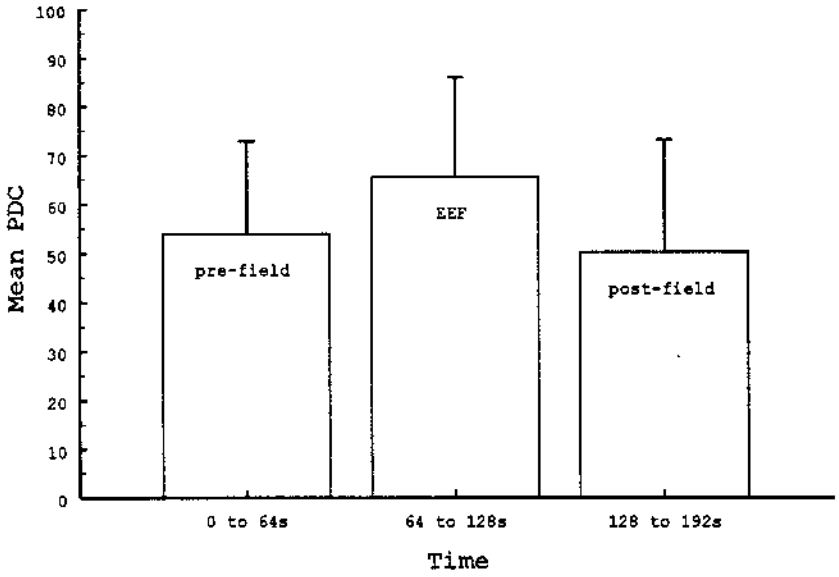

Fig. 7. Effects of a pulsed EEF (20V/cm, 5ms duration, at 1 Hz) on the mean variation in light transmittance (PDC) in 10 isolated crayfish nerves pre-treated with TTX (10mM) for 1 minute.

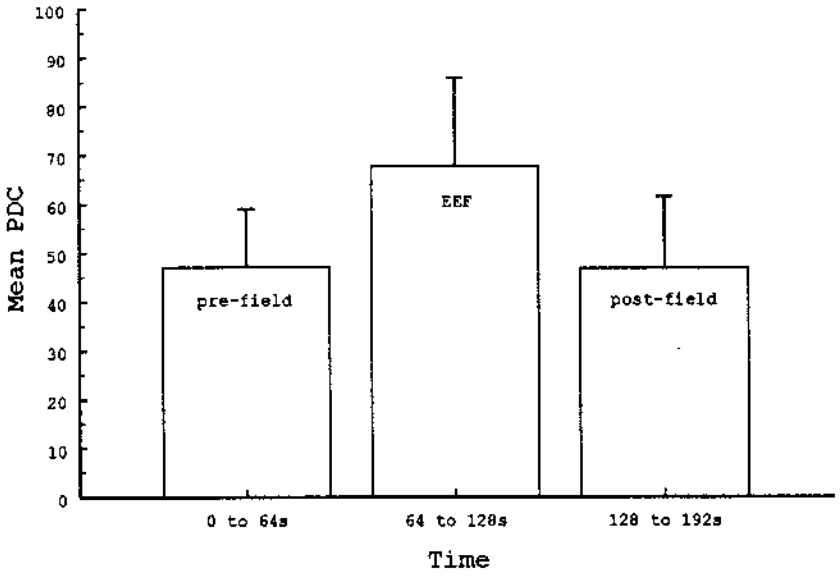

Fig. 8. Effects of an EEF (d.c.: 90V/cm) on the mean variation in light transmittance (PDC) in 10 isolated crayfish nerves.

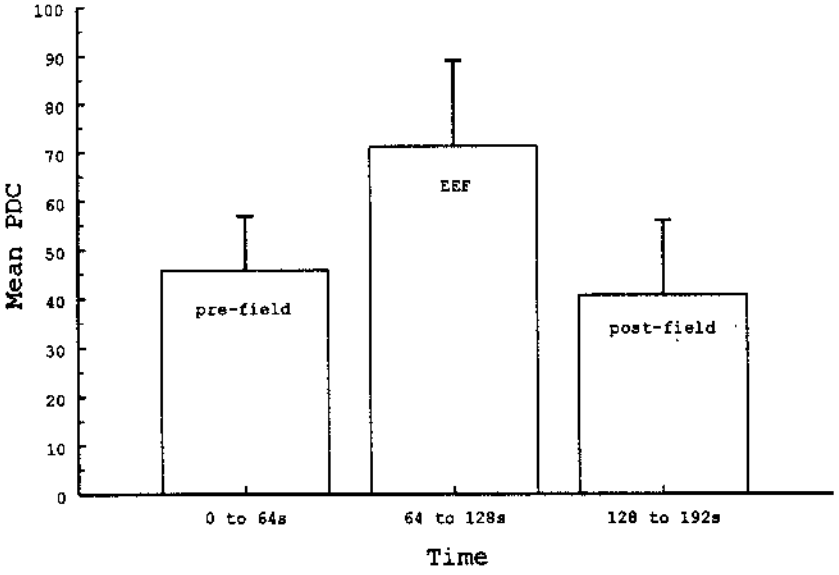

Fig. 9. Effects of an EEF (d.c.: 90V/cm) on the mean variation in light transmittance (PDC) in 10 isolated crayfish nerves pretreated with TTX (10mM) for 1 minute.

transmittance during and after exposure.

(2) Nerves treated with TTX and exposed to the same pulsed field parameters failed to exhibit any statistically significant changes in light transmittance.

(3) Nerves exposed to a d.c. EEF did exhibit statistically significant change in the variation of transmittance during and a decrease in the variation of transmittance following exposure.

(4) Nerves treated with TTX and exposed to a d.c. EEF showed statistically significant changes in the variation in light transmittance during and following exposure.

LIGHT TRANSMITTANCE (PDC)

Condition	Number of Nerves	PDC Pre-exposure t=0 to 64s	PDC EEF exposure t=64 to 128s	PDC Post-exposure t=128 to 192s
Sham Control**	10	57.05 sd=13.78	58.74 sd=11.65	57.92 sd=11.40
TTX-treated (10mM-1min)	10	55.92 sd=10.97	57.09 sd=10.10	56.62 sd=8.91
Pulsed EEF 20 V/cm	10	46.77 sd=20.13	57.29 sd=21.74	60.94 sd=21.92
Pulsed EEF + TTX(10mM)	10	53.90 sd=18.98	65.41 sd=20.50	50.12 sd=22.94
D.C. EEF 20 V/cm	10	47.01 sd=12.00	67.55*** sd=18.15	46.62 sd=14.78
D.C. EEF + TTX(10mM)	10	45.75 sd=11.24	71.13*** sd=17.70	40.5 Sd=15.49

**Sham control: No EEF, No TTX.
*** Statistically significant at $p<0.05$ (Tukey Test).

Table 1: A summary of the effects of EEFs on light transmittance (PDC) on isolated crayfish nerves.

DISCUSSION

Over 100 preliminary experiments were performed to establish the overall experimental protocol used in this study. Some technical difficulties arose during the process of implementing the experiment that deserve attention at this point. First, the signal to noise ratio, as previously mentioned was relatively low as compared with conventional measurements. This was dealt with by finding a window, so to speak, in which maximum changes in light transmittance were apparent. Preliminary studies were used to find the field strength that had maximal effects on the optical properties of the nerves. The most effective d.c. field strength was 90 V/cm. Preliminary pulsed field EEF exposures indicated that the maximal effect was evident at 20 V/cm when measurements were taken within 20ms following a single pulse. Pulsed field strengths between 0.1 V/cm and 100 V/cm failed to exhibit any measurable effects. By lowering environmental sources of electronic interference in ways discussed earlier, it was hoped to have achieved a maximum signal to noise ratio. However, it would be naive to assume that there was absolutely no random fluctuation in the readings. This hopefully was canceled out by the

averaging of 64 signals during the measurement period. Nevertheless, it is still possible that some spikes may have been present in the waveforms that were averaged out. This was the sacrifice that was made in order to ascertain an overall effect. Another parameter of concern was the effect of drying on the nerve. To overcome this, moistened filter paper was used on the inside of the chamber. Nerves still, and probably did, lose water and probably at different rates. The randomness of the population of nerves was counted on to statistically counter that. No nerve was used in any given experiment that did not elicit a compound action potential before and following testing. This was to ensure that the nerve used in an experiment was viable throughout the experiment. Also, the apparatus was constructed with several cannibalized parts of electronic devices. There lies a certain degree of error in that alone. It was attempted to calibrate each step of the experiment with the available equipment so as to ensure accuracy. The light source voltage was regulated, absolute dark was measured against maximal light, and measurements with no nerve present were taken to ensure maximal calibration of the apparatus. Finally, since each experiment contained three phases, each nerve served as its own control (i.e. the pre-exposure period).

The data in Figure 5 indicate relatively no effect of

the Na^+ channel blocker, TTX, on the PDC in the nerves when compared with the PDC in control nerves seen in Figure 4. These findings indicate that if the Na^+ channels were blocked by the TTX, it did not affect the transparency of the nerve.

Most recent studies have pointed to pulsed EEFs as the major progenitor for effects at the membrane level. The data presented here, though not statistically significant, were in agreement with current literature that indicate structural changes in biological membranes in response to pulsed EEFs per se. A change in transparency of the nerve during the period of exposure to the pulsed EEF was sustained into the post-exposure period. Such lingering effects with the pulsed EEF tend to support popular current stipulations that attribute certain long range effects such as cancer growth to pulsed EEFs(14,15). However, these effects were not measured using optical techniques. The nerves treated with TTX and pulsed EEFs did exhibit recovery in the PDC indicating a degree of TTX action.

The data obtained with the d.c. EEFs were pronounced and statistically significant. Nerves exposed to a 90 V/cm d.c. field exhibited a clear change in transparency during exposure that returned to control levels during the post-test period indicating recovery in the affected nerves. Interestingly, TTX appeared to have had an additive effect

on the enhancement in PDC during the exposure period. Such an additive effect of the TTX, however, was not sustained in the post-exposure period.

It is difficult to account for the differences in the data between the d.c. and pulsed field-treated nerves. One possibility might be in the amplitude of the fields, i.e. 90 V/cm d.c. field versus 20 V/cm pulsed field. Another possible explanation of the discrepancy may involve a combination of rapidly reversing (<20ms) cell-surface ion binding effects that were immediately affected by the introduction of a field and were followed by a general electromigration of ions within the entirety of the nerve. The effects of this migration in turn would cause more pronounced changes in transparency. Nerves pre-treated with TTX and then exposed to a d.c. field exhibited an enhanced effect during the exposure period. If there was migration of Na^+ ions across the membrane that directly caused the effect, it should have been diminished by treatment with TTX. It is possible that different processes were involved when the pulsed and d.c. effects are compared. Pulsed EEF effects may have been caused by some kind of adaptive mechanism at the cell surface that involved Na^+ channels and lasted a relatively long period whereas d.c. field effects may have been caused by migration of ions throughout the nerve compartments not just through Na^+ channels. Since the

gated region of measurement was 20 ms following the pulsed EEFs and the d.c. transmittance levels were not gated but represented a continuous measurement, there leaves open the possibility that migration may have occurred during the d.c. field as is suggested by Lee(7) and Yakovlev(8).

In general, the data indicate three possible mechanisms that may have been involved with EEF effects:(1) a change in ion flux,(2) a change in water and/or fluid flux within the various membrane compartments in the nerve, and (3) simply a change in the physical integrity of the protein structures within the compound nerve. Since the potent Na^+ channel blocker TTX failed to noticeably alter the PDC in nerves exposed to both pulsed and d.c. EEFs, it would indicate that changes in the Na^+ channels may not be involved in the EEF effect. However, one might consider another important ion, Ca^{++}, and its role in EEF effects. Adey(23) has written extensively on the effects of EEFs on Ca^{++} flux in and out of nerve cell membranes. He reports that EEFs alter the extracellular binding sites for Ca^{++} that are involved in a mechanism(s) controlling its influx and efflux. Moreover, he states that Ca^{++} is involved with transmembrane signaling that results in the amplification of initially small signals (e.g. EEFs). Such effects could have profound effects on nerve cell functions. If EEF exposure brought about an increase in the ionized form of Ca^{++}, one would expect

immediate changes in the structure of the protein portions of the membranes. This might result in the formation of channels and/or pores that would alter the flux of water and/or ions within the nerve compartments. This, in turn, might alter the PDC. Researchers have hypothesized that energy induced into the interplay of surface channels and their corresponding ions cause increased binding or increased splitting of the surface molecules(3,4,10). At the concentration of TTX used, it can be safely assumed that nearly all of the Na^+ channels were blocked(24). Of course, water moves along with Na^+ because of the relatively large shell of hydration of the ion. If the EEF did produce changes in water movement within the nerve compartments, one would expect swelling or shrinking of neuronal structures which may have been present that might result in a change in light transmittance. Cohen et al.(18) have provided an explanation based on the swelling of the nerve or individual axons within the nerve. Using hypotonic solutions, they were able to recreate changes in light scattering that were seen in the crab nerve during action potentials. Introduction of chloride ions which affected the osmolarity of the intracellular fluid also affected light scattering. One must keep in mind that a compound nerve in a crayfish is a composite of hundreds of axons , periaxonal spaces, perineurium, Schwann cells, myelin sheaths, epineurium, and

related structures. Changes in these structures added to movement of ions and water could cause a variety of subtle EEF responses. The results seen therefore would represent the sum of the effects from all of these sources.

An important point regarding the findings presented here is that if the observed changes that occurred in relatively small field strengths (20-90 V/cm) and for relatively short exposure times (ms), one wonders about the effects of the higher fields measured around and under present-day high power lines (350-1000 KV) and power stations. It would be an interesting endeavor to apply this same study to a single axon such as the squid giant axon. In this way, it would be possible to study the effects on a single membrane bound structure and thus eliminate the influence of other structures.

In summary, the data in this study indicate that (1) one can alter the optic properties , in this case a change in the variation of light transmittance, in a compound nerve with an EEF, (2) such effects occurred at relatively low levels of field strengths and relatively short exposure times, (3) a change in light transmittance in a pulsed EEF was not as pronounced as in a d.c. EEF, (4) the effect was reversible in the d.c. field but not in the pulsed field, (5) the Na^+ channel blocker TTX may have helped reverse the enhancement effect in both pulsed field and d.c. exposed

nerves, moreover, TTX appeared to have an additive effect on the light transmittance in a d.c. field, and (6) the optical change observed was considered to be due to either subtle physical alterations in the myriad of structures within the compound nerve or due to changes in ion and fluid flux along and within the various membrane compartments.

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